

POLAROGRAPHIC STUDIES ON REDOX REACTIVITY OF POLYMER-HEMIN COMPLEX IN  
A HYDROPHOBIC MICROENVIRONMENT

Eishun TSUCHIDA and Kenji HONDA

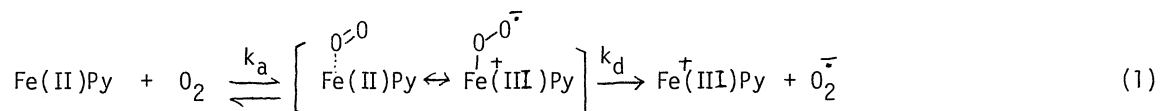
Department of Polymer Chemistry, Waseda University

Nishiohkuho, Shinjuku-ku, Tokyo 160

When hemin was bound to partially quaternized polyvinylpyridine and such additives as sodium chloride, sodium lauryl sulfate and polymethacrylic acid were added, it was possible to lower the redox potential of the central ferric ion of hemin and to control the redox reactivity of hemin arbitrarily owing to the change in the property of microenvironment around hemin.

The present authors have studied the reversible oxygenation of heme bound to the synthetic polymers.<sup>1,2)</sup> The interactions between heme and molecular oxygen in solutions have been studied for a long time,<sup>3-6)</sup> but in the former research a stable oxygen complex was not formed especially in an aqueous solution because of the rapid irreversible oxidation of a central ferrous ion. We have pointed out that it is important to include heme into the hydrophobic cavity in order to stabilize an oxygenated heme and to carry out the reversible oxygen-uptake effectively in an aqueous medium. It was clarified in our study that by binding heme to the hydrophobic cavity formed by macromolecules, the oxygen complex of heme can exist in a stable form even in water at room temperature owing to retardation of the irreversible oxidative decomposition of the oxygen complex.

In this paper, the rate constant ( $k_d$ ) at the decomposition step of the oxygen complex represented by Equation (1) will be correlated to the redox potential of the ferric ion of hemin, and the microenvironment around an active site of hemin will be discussed.



It has been already reported by ourselves<sup>7)</sup> that the half-wave potential of reduction of cupric ion was linearly correlated to the activation energy of the redox reaction between the copper

complex of Schiff base containing a pyrrole ring and 1,1-diphenyl-2-picrylhydrazyl and that the half-wave potential of reduction of the polymer complex was lower than that of the monomeric analog since a central metal ion of the polymer complex is surrounded by the hydrophobic polymer chains and so the microenvironment around the metal ion is non-polar even in water. We have examined in this study what change will happen in the redox potential of a ferric ion of hemin in the case of the polymer-hemin complex, if hemin can be included into the hydrophobic cavity of the polymer chains.

The polymer-hemin complex used here is an iron-protoporphyrin complex bound to partially quaternized polyvinylpyridine (QPVP) at the axial site of hemin-iron. In the complexation between hemin and QPVP, the average coordination number ( $\bar{n}$ ) of axial base and the equilibrium constant ( $K$ ) were as follows:  $\bar{n} = 1.30$ ,  $K = 2.60 \times 10^2$  l/mol, in  $H_2O$ -DMF (9 : 1), at 25°C.

The half-wave potentials of reduction of hemin were measured by Yanaco P-8 Type Polarograph (Yanagimoto Co. Ltd.), the cathode of which is a dropping mercury electrode and the anode of which is a mercury pool. The conditions of measurement are as follows:  $[Hemin] = 1.0 \times 10^{-5}$  mol/l,  $[QPVP] = 1.3 \times 10^{-3}$  mol/l, in  $H_2O$ /DMF (9 : 1). The solution was used as a sample for measurement after saturation of nitrogen gas.

The rate constant ( $k_d$ ) at the decomposition step of the oxygen complex was determined from the first order plots of the decay of absorbance at 402 nm due to the oxygen complex during the reaction of the reduced hemochrome with oxygen in an aqueous solution.

When QPVP-hemichrome was dissolved in the mixture of  $H_2O$ -DMF (9 : 1) and sodium chloride was added, the polymer chains of QPVP were increasingly shrunk with increasing the concentration of NaCl added, and correspondingly the half-wave potential ( $E_{1/2}$ ) of reduction of ferric ion of QPVP-hemichrome was lowered. Figure 1 shows that the rate constant of  $k_d$  decreased as  $E_{1/2}$  of QPVP-hemichrome decreased. The half-wave potential of reduction of the monomeric pyridine-hemichrome was always equal to  $-0.20 \pm 0.01$  V against the concentration of NaCl added. It was thus elucidated that the redox potential of the metal ion surrounded by polymer chains is lowered due to the increment in hydrophobicity around the metal ion.

When polymethacrylic acid (PMAA) was added instead of NaCl, PMAA interacted with QPVP through the electrostatic force to form the polyion complex. Perhaps hemin may be surrounded by the hydrophobic environment of the resulting polyion complex and this is considered to be responsible for a lowering of both  $E_{1/2}$  and  $k_d$ , as Figure 2 shows. Figure 3 shows the effect of the globular micell of sodium lauryl sulfate (NaLS) on the monomeric pyridine-hemichrome system. That is, at the higher level of the critical micell concentration of NaLS, the pyridine-hemichrome was observed to be included into the hydrophobic globular micell by the spectroscopy and then  $E_{1/2}$  decreased drastically accompanying the decrease in  $k_d$  due to the same reason of the polymeric system.

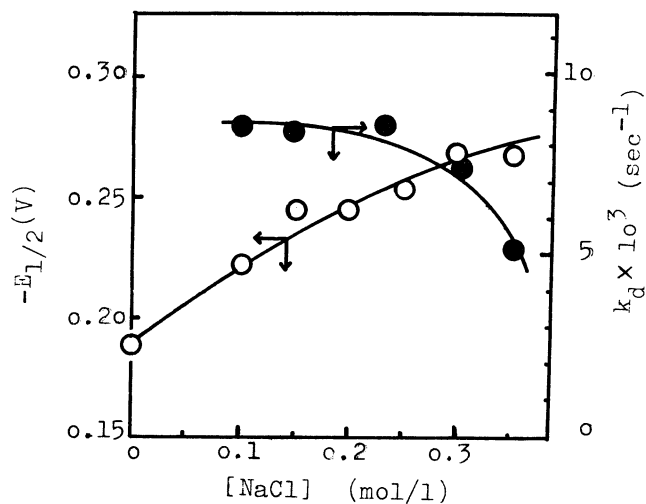


Fig. 1 Relationship between Half-wave Potential of Reduction of Polymeric Hemichrome and Concentration of NaCl Added  
 $[\text{Hemin}] = 1.0 \times 10^{-5} \text{ mol/l}$ ,  $[\text{QPVP}] = 1.0 \times 10^{-2} \text{ mol/l}$  ( $Q\% = 59.2$ ,  $\overline{Pn} = 49$ ) in  $\text{H}_2\text{O}/\text{DMF} = 9/1$

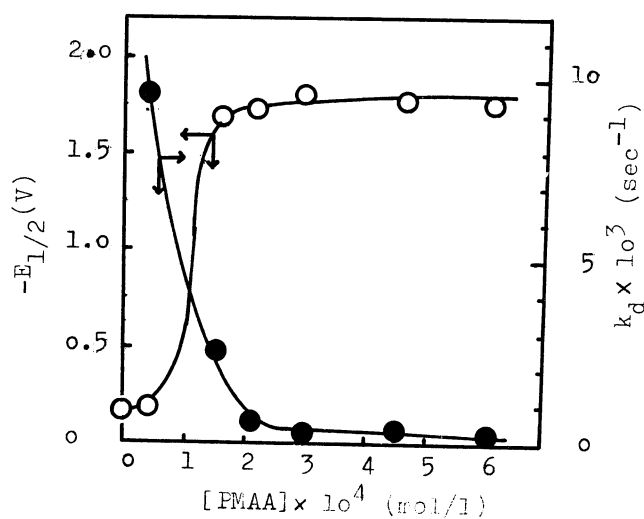


Fig. 2 Relationship between Half-wave Potential of Reduction of Polymeric Hemichrome and Concentration of PMAA Added  
 $[\text{Hemin}] = 1.0 \times 10^{-5} \text{ mol/l}$ ,  $[\text{QPVP}] = 1.3 \times 10^{-3} \text{ mol/l}$  ( $Q\% = 23.1$ ,  $\overline{Pn} = 49$ ),  $\overline{Pn}_{(\text{PMAA})} = 200$ , in  $\text{H}_2\text{O}/\text{DMF} = 9/1$

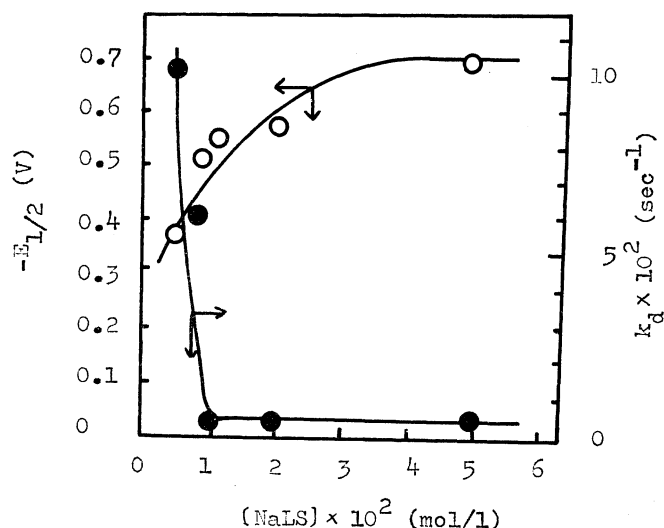


Fig. 3 Relationship between Half-wave Potential of Reduction of Monomeric Hemichrome and Concentration of NaLS Added

[Hemin] =  $1.0 \times 10^{-5}$  mol/l, [Py] =  $1.0 \times 10^{-2}$  mol/l in  $H_2O/DMF = 9/1$

In conclusion, when hemin was successfully situated in the hydrophobic cavity of polymer chains, the redox potential of a central ferric ion of hemin was lowered and so the decomposition of the oxygen complex of heme could be retarded even in an aqueous solution at room temperature, so that the resulting oxygen complex could exist in a very stable form.

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